Curso de Aplicaciones clínicas de la Citometria de Flujo

Estudio de citopenias y sindromes mielodisplásicos

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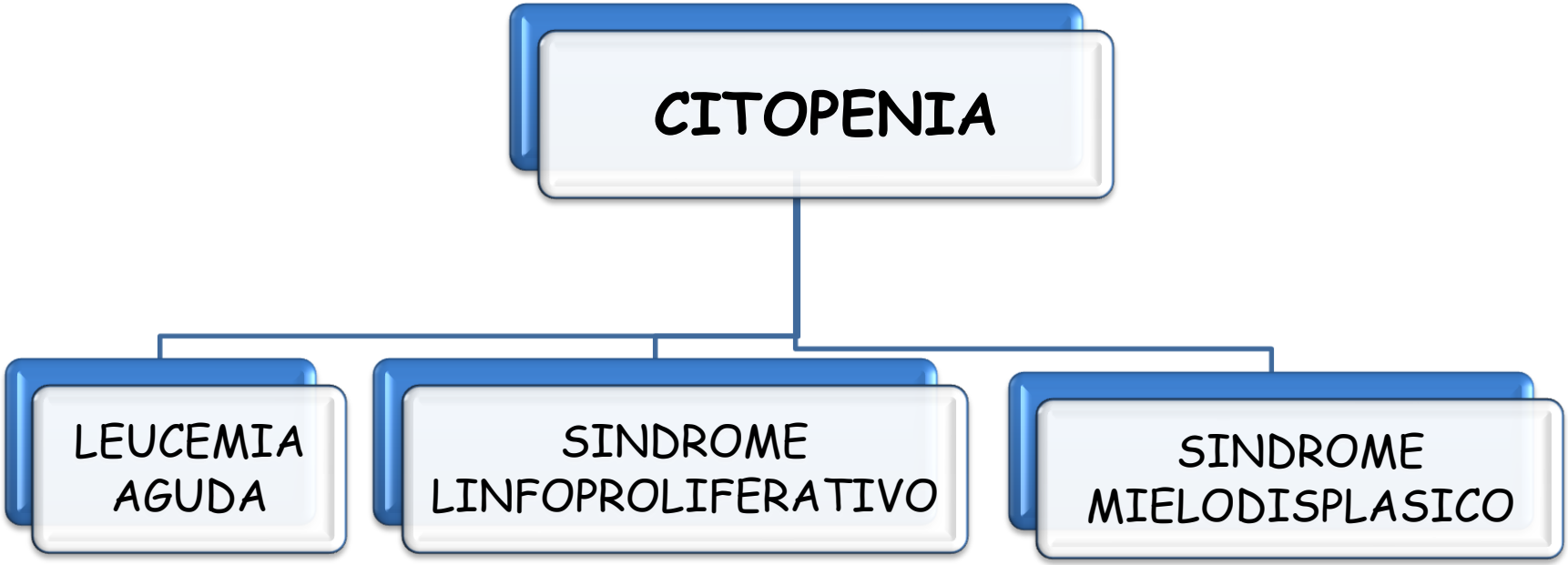
CITOPENIA
LEUCEMIA AGUDA
SINDROME LINFOPROLIFERATIVO
SINDROME MIELODISPLASICO

Sindrome Mielodisplásicos
Definición

Los Sindromes Mielodisplásicos son un grupo heterogéneo de enfermedades clonales de las células progenitoras hematopoyéticas de la médula ósea caracterizada por una hematopoyesis inefectiva con alteraciones funcionales y morfológicas de los progenitores, desarrollo de citopenias periféricas y aumento del riesgo de evolucionar a leucemia mieloide aguda.

Presencia de alteraciones morfológicas, acumulación de anomalías genéticas y diversas alteraciones fenotípicas en precursores y células maduras en médula ósea.

Blastos < 20%

Generalidades

- Incidencia: 3-5 casos /100.000 habitantes. Su incidencia aumenta con la edad. En > 70 años: 20 casos/100.000 habitantes.
- Edad mediana al momento del diagnóstico entre los 65- 70 años.
- Es más frecuente en hombre que en mujeres.
- Poco frecuente en niños y adolescentes. Representa < 5% de los tumores hematopoyéticos en pacientes menores de 14 años.
- El pronóstico y el curso de la enfermedad varía entre pacientes.
- Existen SMD primarios y secundarios.

Causas de SMD secundarios

- Agentes alquilantes: mostazas nitrogenadas, nitrosureas, clorambucilo, ciclofosfamida, cisplatino y carboplatino.
- Agentes que inhiben la DNA topoisomerasa II: etopósidos y antracíclicos.
- Radioterapia: irradiación corporal total o hemicuerpo
- Exposición a solventes, químicos, pesticidas, fertilizantes, benceno y derivados del petróleo.

Criterios diagnósticos

(A) Pre-requisitos esenciales

1. Al menos una *citopenia persistente:* Hb <11 g/dL (La clasificación de la OMS 2016 propone disminuir el límite en 10 g/dL); neutrófilos <1,8 x 10^9/L o plaquetas <100 x 10^9/L (Se debe tener en consideración los factores propios del paciente, como las características étnicas, altitud de su lugar de residencia y los valores de referencia definidos por el laboratorio local).
2. Exclusión de otras enfermedades, hematológicas o no, como causa primaria (Tabla 1).

(B) Criterios decisivos

1. Displasia en al menos 10% de las células en al menos una de las líneas celulares en médula ósea: eritroide (o >15% de sideroblastos en anillo), granulocítica y/o megacariocítica.
2. Porcentaje de blastos en el aspirado medular entre 5-19%.
3. Anomalías cromosómicas recurrentes características (por citogenética, Tabla 7).

(C) Co-criterios

1. Inmunofenotipo anormal identificado por citometría de flujo en células de MO con múltiples aberraciones asociadas con un fenotipo de SMD (Tabla 5).
2. Evidencia molecular de monoclonalidad diagnosticado por ensayo HUMARA o técnicas de "microarrays", o presencia de mutaciones puntuales características.
3. Hallazgos anormales en la histopatología de la MO (>10% de micromegacariocitos displásicos detectados por inmunohistoquímica, localización anormal de progenitores, cluster de CD34+)

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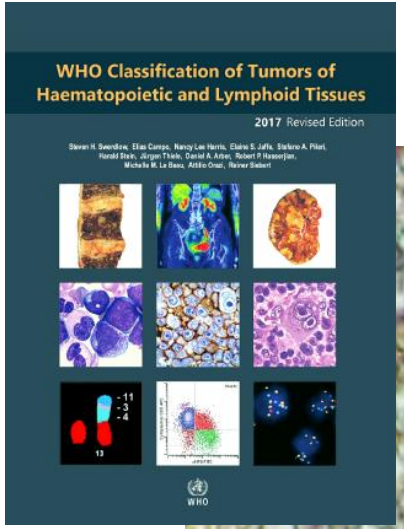
Sociedad Argentina de Hematologìa. Guias de Diagnóstico y Tratamiento. 2019.

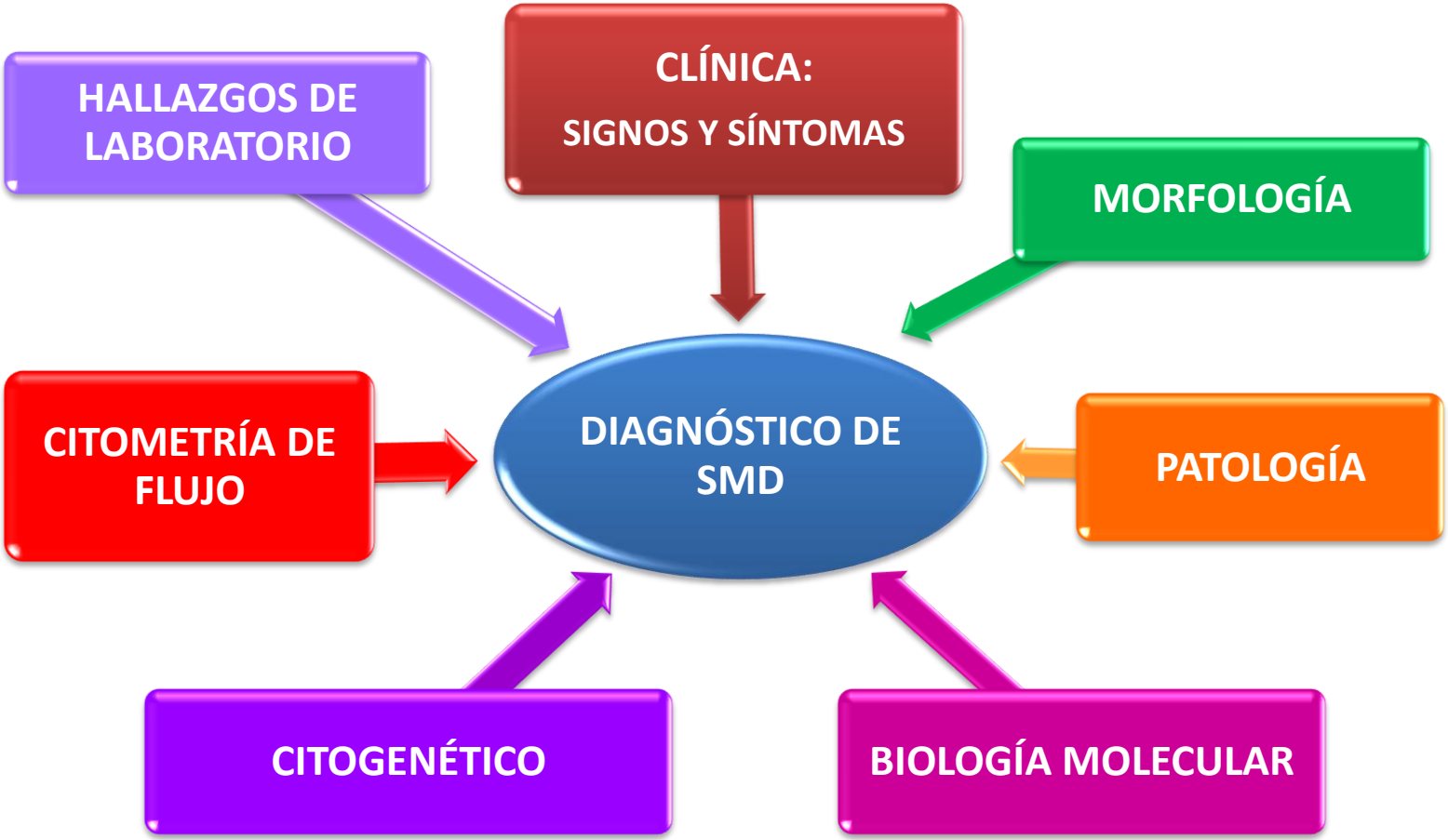
Clasificación WHO



CHAPTER 6

Myelodysplastic syndromes

Myelodysplastic syndrome with single lineage dysplasia
Myelodysplastic syndrome with ring sideroblasts
Myelodysplastic syndrome with multilineage dysplasia
Myelodysplastic syndrome with excess blasts
Myelodysplastic syndrome with isolated del(5q)
Myelodysplastic syndrome, unclassifiable
Childhood myelodysplastic syndrome
Refractory cytopenia of childhood


HALLAZGOS DE LABORATORIO
CLÍNICA:
SIGNOS Y SÍNTOMAS
MORFOLOGÍA
CITOMETRÍA DE FLUJO
DIAGNÓSTICO DE SMD
PATOLOGÍA
CITOGENÉTICO
BIOLOGÍA MOLECULAR

Citometría de Flujo en SMD

- Permite realizar una evaluación cuali-cuantitativa de las poblaciones celulares presentes en la médula ósea detectando anomalías/alteraciones en la expresión antigénica en la diferenciación/maduración celular (hematopoyesis alterada).

- En casos con cambios displásicos mínimos por morfología, porcentaje de blastos en rango normal o cariotipo sin alteraciones, la CF puede ser una herramienta diagnóstica de utilidad para distinguir SMD de otras citopenias no clonales.

- La CF siempre debe interpretarse en forma integrada junto con la citomorfología, citogenética e histopatología.

- La CF es un co-criterio diagnóstico en SMD.

IMPORTANTE → OPTIMIZAR Y ESTANDARIZAR

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SPECIAL REPORT

Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group

TM Westers[1], R Ireland[2,24], W Kern[3,24], C Alhan[1], JS Balleisen[4], P Bettelheim[5], K Burbury[6], M Cullen[7], JA Cutler[8], MG Della Porta[9], AM Dräger[1], J Feuillard[10], P Font[11], U Germing[4], D Haase[12], U Johansson[13], S Kordasti[2], MR Loken[8], L Malcovati[9], JG te Marvelde[14], S Matarraz[15], T Milne[2], B Moshaver[16], GJ Mufti[2], K Ogata[17], A Orfao[15], A Porwit[18], K Psarra[19], SJ Richards[7], D Subirá[20], V Tindell[2], T Vallespi[21], P Valent[22], VHJ van der Velden[14], TM de Witte[16], DA Wells[8], F Zettl[12], MC Béné[23] and AA van de Loosdrecht[1,24]

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ORIGINAL ARTICLE

EuroFlow antibody panels for standardized *n*-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes

JJM van Dongen[1], L Lhermitte[2], S Böttcher[3], J Almeida[4], VHJ van der Velden[1], J Flores-Montero[4], A Rawstron[5], V Asnafi[2], Q Lécrevisse[4], P Lucio[6], E Mejstrikova[7], T Szczepański[8], T Kalina[7], R de Tute[5], M Brüggemann[3], L Sedek[8], M Cullen[5], AW Langerak[1], A Mendonça[6], E Macintyre[2], M Martin-Ayuso[9], O Hrusak[7], MB Vidriales[10] and A Orfao[4] on behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708)

Objetivo de los Consensos

- Estandarizar el estudio de esta patología para poder llegar a resultados homogéneos

- Unificar criterios pre-analíticos y procesamiento de la muestra. Definir paneles de marcación mínimos y métodos de análisis. Establecer modelos de informes de resultados.

- Educación y capacitación continua.

Available online at www.sciencedirect.com
ScienceDirect
Leukemia Research 31 (2007) 727–736
Leukemia Research
www.elsevier.com/locate/leukres

Consensus report

Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: Consensus statements and report from a working conference

Peter Valent[a,*], Hans-Peter Horny[b], John M. Bennett[c], Christa Fonatsch[d], Ulrich Germing[e], Peter Greenberg[f], Torsten Haferlach[g], Detlef Haase[h], Hans-Jochen Kolb[i], Otto Krieger[j], Michael Loken[k], Arjan van de Loosdrecht[l], Kiyoyuki Ogata[m], Alberto Orfao[n], Michael Pfeilstöcker[o], Björn Rüter[p], Wolfgang R. Sperr[a], Reinhard Stauder[q], Denise A. Wells[k]

- Define criterios mínimos de diagnóstico en SMD.
- Recomendaciones para morfología.
- Recomendaciones para histología e inmunohistoquímica de MO.
- Recomendaciones para el estudio de cariotipo.
- Análisis molecular en SMD.
- Estudio por citometría de flujo.
- Recomendaciones terapéuticas.

Table 4
Recurrent phenotypic abnormalities detected by flow cytometry in MDS

CD34+ myeloid progenitors
Absolute and relative increase in CD34+ cells
Expression of CD11b[a] and/or CD15
Lack of expression of CD13, CD33, or HLA-DR
Expression of 'lymphoid' antigens: CD5, CD7, CD19, or CD56
Decreased CD45 expression
Abnormal increased or decreased intensity of CD34
Abnormally decreased CD38 expression
CD34+ B cell progenitors (CD34+/CD10+)
Absolute and relative (to all CD34+) decrease in CD34+/CD10+ cells
Maturing myeloid (neutrophil) cells
Hypogranularity as evidenced by decreased right angle light scatter
Abnormalities in relationship patterns of myeloid antigens
Asynchronous maturation
Lack of expression of CD13 or CD33
Expression of CD34
Expression of 'lymphoid' antigens
Decreased CD45 expression
Monocytes
Abnormalities in relationship patterns of HLA-DR, CD11b, CD13, CD14, CD33
Lack of expression of CD13, CD14, CD16, or CD33
Expression of CD34
Expression of 'lymphoid' antigens (with the exception of CD4)
Erythroid precursor cells
Abnormal expression of CD45
Expression of CD34
Abnormal expression of CD71, CD117, or CD235a

[a] *Note:* (immature) basophils may also co-express some CD34 with CD11b.

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DECISION MAKING AND PROBLEM SOLVING

Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometric in myelodysplastic syndromes

Arjan A. van de Loosdrecht,[1] Canan Alhan,[1] Marie Christine Béné,[2] Matteo G. Della Porta,[3] Angelika M. Dräger,[1] Jean Feuillard,[4] Patricia Font,[5] Ulrich Germing,[6] Detlef Haase,[7] Christa H. Homburg,[8] Robin Ireland,[9] Joop H. Jansen,[10] Wolfgang Kern,[11] Luca Malcovati,[3] Jeroen G. te Marvelde,[12] Ghulam J. Mufti,[9] Kiyoyuki Ogata,[13] Alberto Orfao,[14] Gert J. Ossenkoppele,[1] Anna Porwit,[15] Frank W. Preijers,[10] Stephen J. Richards,[16] Gerrit Jan Schuurhuis,[1] Dolores Subirá,[17] Peter Valent,[18] Vincent H.J. van der Velden,[12] Paresh Vyas,[19] August H. Westra,[1] Theo M. de Witte,[10] Denise A. Wells,[20] Michael R. Loken,[20] and Theresia M. Westers[1]

- Preparación, conservación y procesamiento de la muestra.
- Parámetros aberrantes hallados en las distintas poblaciones de la MO.
- Combinaciones de Acs recomendadas (4 colores).
- Análisis de los distintos compartimientos de la MO.

Haematológica, 2009

Table 1. Parameters scored as aberrant in the immature compartment.

Myeloblasts	%[1]	B cell progenitors	%
Increased percentage[2]	91	Decreased number/percentage[†] increased number/percentage	82 45
Abnormal granularity (SSC)[2]	73		
Abnormal expression of CD45[2]	100		
Abnormal expression of CD13	100		
Abnormal expression of CD33	100		
Abnormal expression of CD34[2]	91		
Abnormal expression of CD117[2]	82		
Expression of CD11b[2]	82		
Expression of CD15[2]	64		
Abnormal expression of HLA-DR[2]	55		
Expression of CD36	45		
Abnormal expression of TdT	36		
Expression of CD41/CD42b/CD61	36		
Expression of CD38	27		
Expression of CD4	18		
Expression of lineage infidelity marker CD2[2]	82		
Expression of lineage infidelity marker CD5[2]	82		
Expression of lineage infidelity marker CD7[2]	91		
Expression of lineage infidelity marker CD19[2]	91		
Expression of lineage infidelity marker CD56[2]	91		

[1]percentage of survey participants who analyzed a given aberrancy; [2]considered as relevant finding.

Table 2. Parameters scored as aberrant in the maturing myeloid and monocytic compartment.

Granulocytes (maturing myeloid cells)	%[1]	Monocytes	%[1]
Decreased myeloid *vs.* lymphoid ratio (<1)	36	Decreased/increased as compared to lymphocytes[2]	45
Abnormal granularity (SSC)[2]	100	Abnormal granularity (SSC)	45
Abnormal expression of CD45[2]	64	Abnormal expression of CD45	55
Asynchronous shift to the left	82	Abnormal distribution of immature/mature cells	
Abnormal CD16/CD13 pattern[2]	82	Abnormal expression of CD33[2]	73
Abnormal CD11b/CD13 pattern[2]	73	Expression of HLA-DR	73
Abnormal expression of CD33	73	Abnormal CD11b/HLA-DR pattern[2]	73
Expression of CD34[2]	73	Expression of CD34	73
Expression of HLA-DR	73	Abnormal expression of CD14	73
Abnormal expression of CD15	55	Abnormal expression of CD13[2]	73
Abnormal/lack of expression of CD10	45	Abnormal expression of CD36[2]	45
Abnormal expression of CD36	45	Abnormal expression of CD64	36
Abnormal expression of CD64[2]	36	Abnormal expression of CD11c	18
Abnormal expression of CD66	27	Abnormal expression of CD15	9
Abnormal expression of CD16	9		
Expression of lineage infidelity marker CD2[2]	73	Expression of lineage infidelity marker CD2[2]	73
Expression of lineage infidelity marker CD5	73	Expression of lineage infidelity marker CD5[2]	73
Expression of lineage infidelity marker CD7[2]	82	Expression of lineage infidelity marker CD7[2]	73
Expression of lineage infidelity marker CD19[2]	82	Expression of lineage infidelity marker CD19[2]	73
Overexpression of CD56	45	Overexpression of CD56[2]	91

[1]percentage of survey participants who analyzed a given aberrancy; [2]considered as relevant finding.

Table 3. Parameters scored as aberrant in the erythroid compartment.

Erythroid cells	%[1]
Increased percentage after lysis	45
Abnormal granularity (SSC)	45
Abnormal expression of CD45	64
Abnormal expression of CD71	73
Abnormal CD71/CD235a pattern[2]	64
Abnormal expression of CD235a	55
Abnormal expression of CD117	55
Abnormal expression of CD34	45
Abnormal expression of CD36	36
Abnormal expression of CD105[2]	36
Abnormal expression of H-ferritin	18
Abnormal expression of M-ferritin	18

[1]percentage of survey participants who analyzed a given aberrancy; [2]considered as relevant finding.

Table 5. Example of a screening panel for 4-color flow cytometric of dysplasia in myelodysplastic syndromes.

	FL-1	FL-2	FL-3	FL-4
1			CD45	
2	CD71	CD235a	CD45	CD117
3	CD36	CD64	CD45	CD14
4	CD10	CD33	CD45	CD14
5	CD16	CD13	CD45	CD11b
6	HLA-DR	CD117	CD45	CD11b
7	CD13+CD33	CD117	CD45	CD34
8	CD13	CD7	CD45	CD34
9	CD2	CD56	CD45	CD34
10	CD5	CD19	CD45	CD34
11	CD15	CD11b	CD45	CD34

PRIMER CONSENSO DEL GRCF PARA EL ESTUDIO DE SINDROMES MIELODISPLASICOS POR CITOMETRIA DE FLUJO

- Recomendaciones técnicas generales.
  - Tipo de muestra y conservación.
  - Procesamiento de la muestra.
  - Adquisición.
  - Paneles recomendados (3 y 4 fluorocromos).
- Recomendaciones para el análisis.
- Recomendaciones sobre el informe.

2010

Recomendación de paneles

4 colores mínimo. Panel de 8 colores de Euroflow

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- Requerimientos mínimos recomendados para el estudio de SMD por CF.
- Recomendaciones sobre el procesamiento de la muestra.
- Propuesta de Acs para evaluar las distintas poblaciones de la MO.
- Análisis e identificación de aberrancias en las poblaciones a estudiar.

Table 2. Processing of bone marrow samples for flow cytometric analysis of dysplasia

	Recommendations[a]
Anticoagulant in bone marrow sample	Preferably heparin (alternative: EDTA)
Storage temperature	Room temperature
Processing time	< 24 h
Pre-treatment before staining (optional[b])	Bulk-lysis of erythrocytes by ammoniumchloride (without a fixative)
Washing buffer (optional)	Phosphate-buffered saline with 0.5% bovine or human serum albumin
Staining	Minimal four-color flow cytometry Minimum of 500000 cells incubated per antibody combination
Preservation of stable antigen expression after staining	Paraformaldehyde fixation (0.5%)
Gating for immature myeloid cells	Called progenitors rather than blasts
Doublets	To be excluded by a FSC height/FSC area graph
Degranulated neutrophils	Assessed by comparing their SSC (in linear or logarithmic scale) to that of lymphocytes; of note, these cells will overlap with progenitors and/or monocytes and must be identified by appropriate markers
Glycosyl-phospatidyl-inositol-anchored antigens (i.e., CD14, CD16, CD24)	Expression will be altered if PNH is present, but this does not impair MDS diagnosis

Abbreviations: ELN, European LeukemiaNet; MDS, myelodysplastic syndrome; PNH, paroxysmal nocturnal hemoglobinuria. [a]Summary of ELN recommendations as published in 2009.[6] [b]A stain/lysis no wash procedure can also be used, though care must be taken when interpreting expression of markers that are present on mature erythrocytes, such as glycophorin A.

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Table 1. Recommended minimal requirements to assess dysplasia by flow cytometry

Bone marrow subset	*Recommended analyses*	*Aberrancy*
Immature myeloid and monocytic progenitors	Percentage of cells in nucleated cell fraction[a]	Increased percentage
	Expression of CD45	Lack of/decreased/increased
	Expression of CD34	Lack of/decreased/increased
	Expression of CD117	Homogenous under/overexpression
	Expression of HLA-DR	Lack of/increased expression
	Expression of CD13 and CD33	Lack of/decreased/increased
	Asynchronous expression of CD11b, CD15	Presence of mature markers
	Expression of CD5, CD7, CD19, CD56[b]	Presence of lineage infidelity markers
Maturing neutrophils	Percentage of cells as ratio to lymphocytes	Decreased
	SSC as ratio vs SSC of lymphocytes	Decreased
	Relationship of CD13 and CD11b	Altered pattern[c]
	Relationship of CD13 and CD16	Altered pattern[c]
	Relationship of CD15 and CD10	Altered pattern[c]; for example, lack of CD10 on mature neutrophils
Monocytes	Percentage of cells	Decreased/increased
	Distribution of maturation stages	Shift towards immature
	Relationship of HLA-DR and CD11b	Altered pattern[c]
	Relationship of CD36 and CD14	Altered pattern[c]
	Expression of CD13 and CD33	(Homogenous) under/overexpression
	Expression of CD56[b]	Presence of lineage infidelity marker
Progenitor B cells	Enumeration as fraction of total CD34+ based on CD45/CD34/SSC in combination with CD10 or CD19	Decreased or absent
Erythroid compartment[d]	Percentage of nucleated erythroid cells	Increased
	Relationship CD71 and CD235a	Altered pattern[c]
	Expression of CD71	Decreased
	Expression of CD36	Decreased
	Percentage of CD117-positive precursors	Increased

[a]Discrepancies in counts between several definitions indicate aberrancies. [b]To be used with caution, as CD56 can be upregulated upon activation, be aware of normal cut-off values (also in stressed marrow). [c]Altered patterns can include altered distribution of maturation stages and/or altered expression levels of indicated antigens. [d]Under evaluation. Examples of several flow cytometric aberrancies in myelodysplastic syndrome can be found on the European LeukemiaNet website: www.leukemia-net.org.

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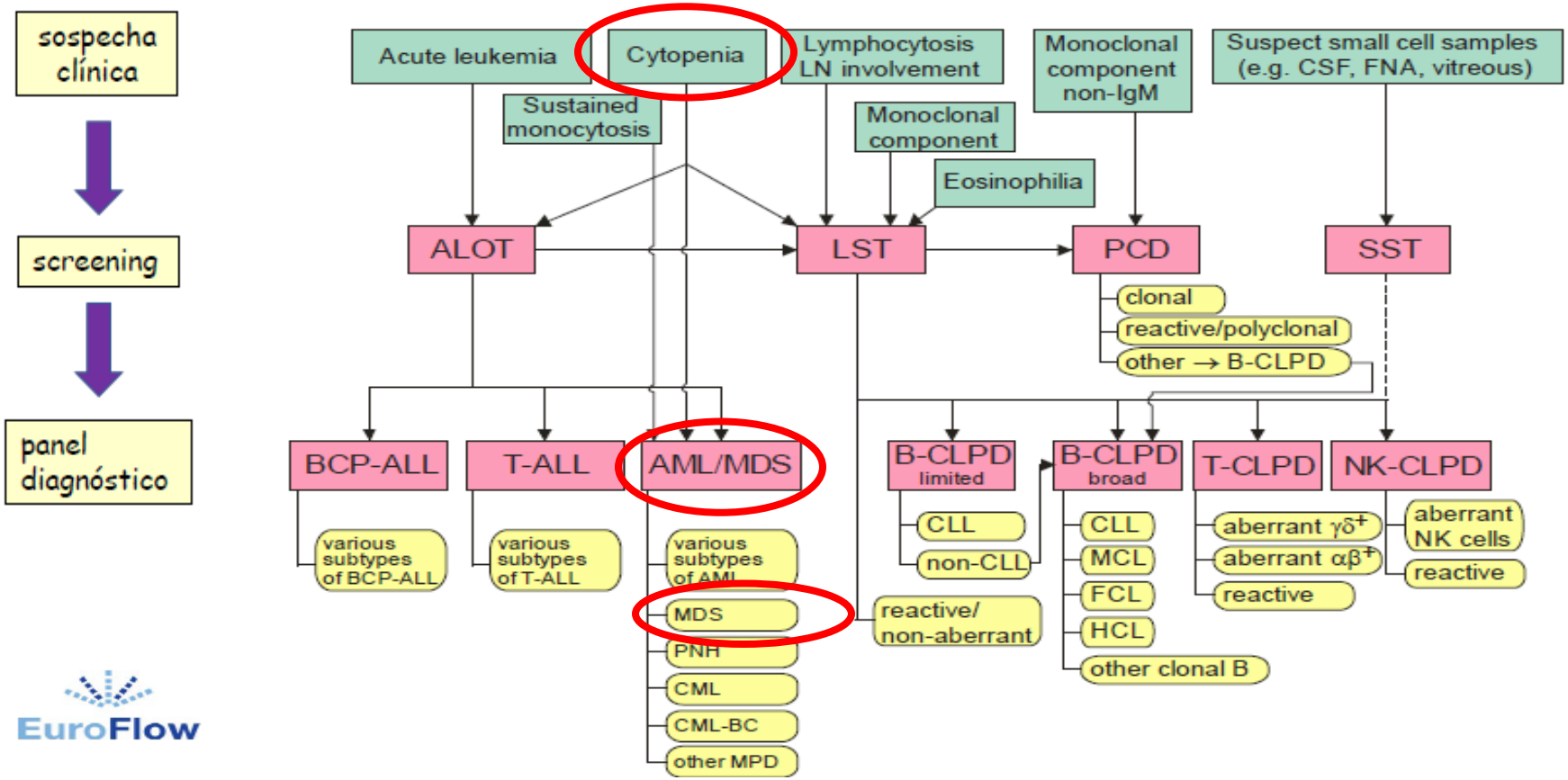
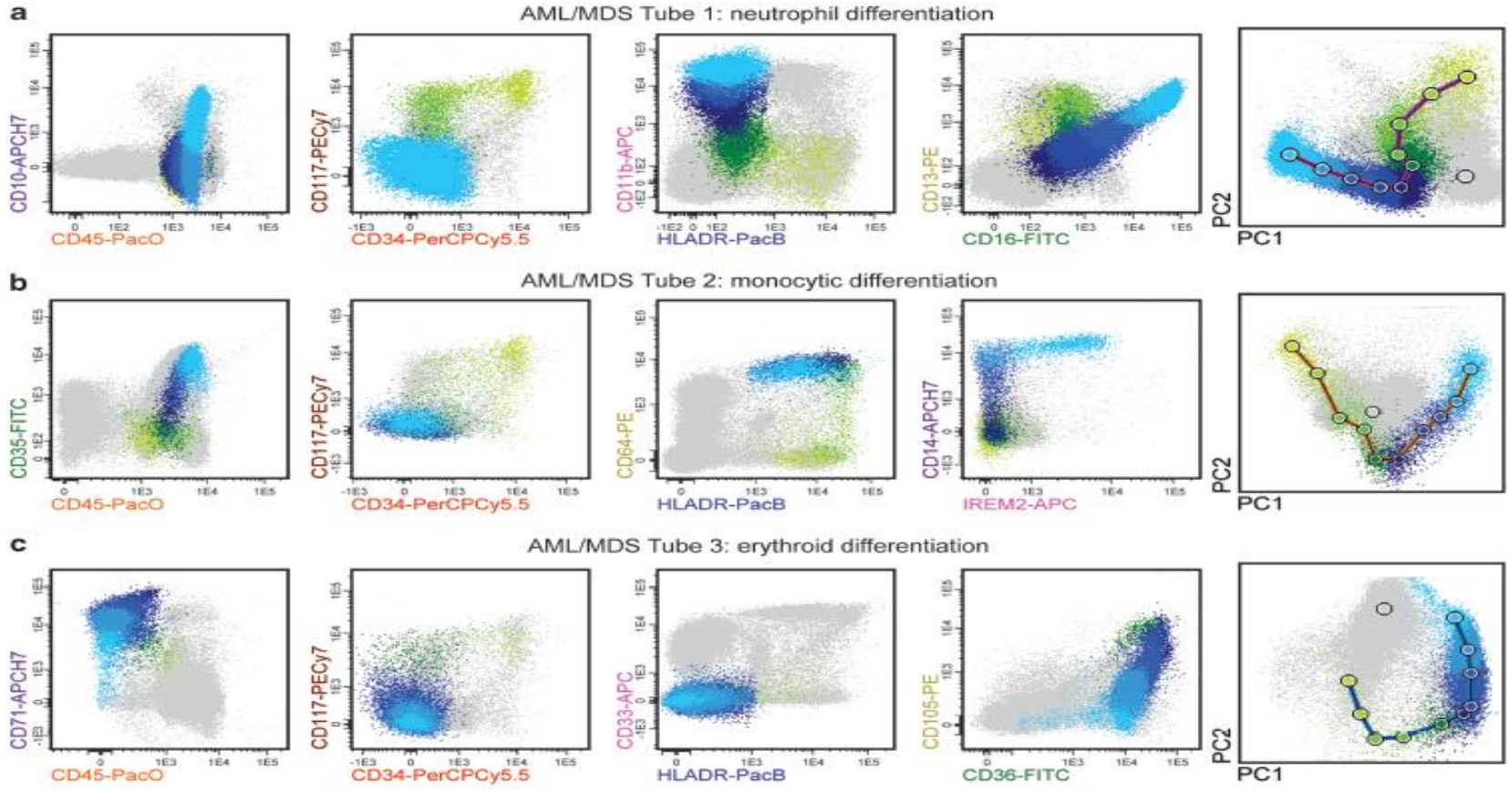
- Establece procedimientos standares para el seteo del citómetro.
- Propone paneles de Anticuerpos de 8 colores según la sospecha clínica.
- Técnicas de marcación estandarizadas.
- Desarrollo de software de análisis: Infinicyt

EUROFLOW: ALGORRITMO DIAGNÓSTICO



Panel LMA/SMD de 8 colores (Euroflow)

PacB	PacO	FITC	PE	PerCPCy5.5	PECy7	APC	APCH7	
HLA-DR	CD45	CD16	CD13	CD34	CD117	CD11b	CD10	Maduración neutrófilo y PNH.
HLA-DR	CD45	CD35	CD64	CD34	CD117	IREM2	CD14	Maduración monocito y PNH
HLA-DR	CD45	CD36	CD105	CD34	CD117	CD33	CD71	Maduración eritroide
HLA-DR	CD45	TDT	CD56	CD34	CD117	CD7	CD19	Maduración B y aberrante Ags linfoides
HLA-DR	CD45	CD15	NG2	CD34	CD117	CD22	CD38	Expresión aberrante en stem cells
HLA-DR	CD45	CD42a CD61	CD203c	CD34	CD117	CD123	CD4	Linaje megacariocítico, basofilos y células dendríticas plasmocitoides.
HLA-DR	CD45	CD41	CD25	CD34	CD117	CD42b	CD9	Caracterización e leucemia megacarioblástica ymastocitosis sistémica.


a
AML/MDS Tube 1: neutrophil differentiation
CD10-APCH7
CD45-PacO
CD117-PECy7
CD34-PerCPCy5.5
CD11b-APC
HLADR-PacB
CD13-PE
CD16-FITC
PC2
PC1
b
AML/MDS Tube 2: monocytic differentiation
CD35-FITC
CD45-PacO
CD117-PECy7
CD34-PerCPCy5.5
CD64-PE
HLADR-PacB
CD14-APCH7
IREM2-APC
PC2
PC1
c
AML/MDS Tube 3: erythroid differentiation
CD71-APCH7
CD45-PacO
CD117-PECy7
CD34-PerCPCy5.5
CD33-APC
HLADR-PacB
CD105-PE
CD36-FITC
PC2
PC1

Citometría de Flujo en SMD

- **Evaluación cuantitativa y cualitativa de:**
  - Precursores CD34+
  - Serie granulocítica neutrófilo
  - Serie monocítica
  - Serie eritroide

- **Evaluación CUANTITATIVA** de precursores CD34(+) y CD117(+)CD34(-), hematogonias B CD34(+) y células monocíticas inmaduras.

- **Evaluación CUALITATIVA** de alteraciones en los patrones de maduración de las distintas series y precursores.

- No existen marcadores ni patrones aberrantes ESPECIFICOS de SMD

Tipo de Muestra

- **Muestra válida**: Aspirado de médula ósea en fresco sin fijador.
- **Anticoagulante:** EDTA (dentro de 24 hs.) o Heparina (más de 24 hs.)
- **Conservación**: temperatura ambiente.
- **Tener en cuenta**:
  - Hemólisis.
  - Presencia de coágulos
  - Hemodilución (volumen de la muestra inferior a 3 ml)
  - Viabilidad celular: depende de anticoagulante utilizado, tiempo y temperatura hasta / durante el procesamiento, métodos de procesamiento, ciertas condiciones que aceleren la muerte celular (terapia recibida, tumor con alta tasa de proliferación).

Alteraciones del Inmunofenotipo

- **Alteraciones fenotípicas:** cambios en la expresión de un Ag con respecto a la normalidad. Ej: aumento, disminución de la intensidad de expresión.

- **Fenotipo aberrante:** expresión de Ags de otro linaje. Ej: expresión de CD56 o CD5 o CD19 en monocitos o granulocitos.

- **Asincronismo madurativo:** Expresión antigénica que no corresponden a ese estado madurativo. Ej: expresión de CD117 en granulocitos.

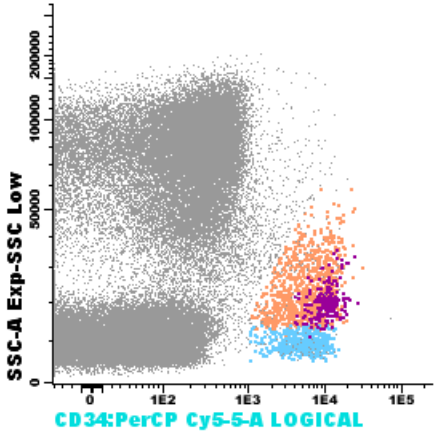
- **Bloqueo o freno madurativo:** Interrupción en el patrón de maduración normal. Ej: patrón de maduración CD11b/CD13 en serie granulocítica.

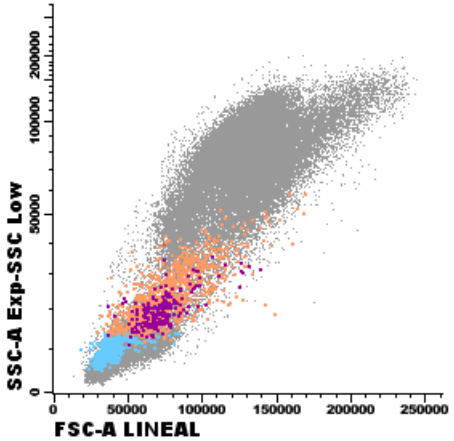
- **Alteración en el FSC/SSC:** Ej: hipogranularidad en granulocitos.

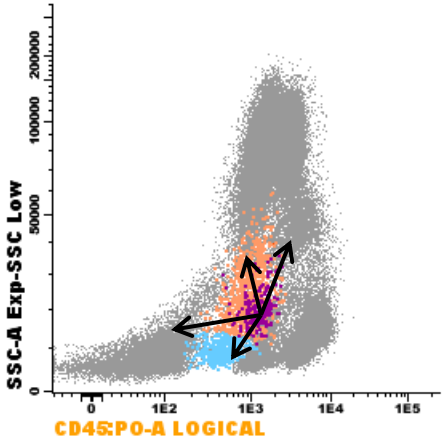
Evaluación de Células Precursoras

Precursores CD34(+)

a- Se seleccionan las células en Gate CD34+ ➔ CD34/SSC
b- En un segundo paso, se excluyen las células NO viables en FSC/SSC.
c- El perfil de CD45/SSC permite constatar pureza de la población seleccionada.

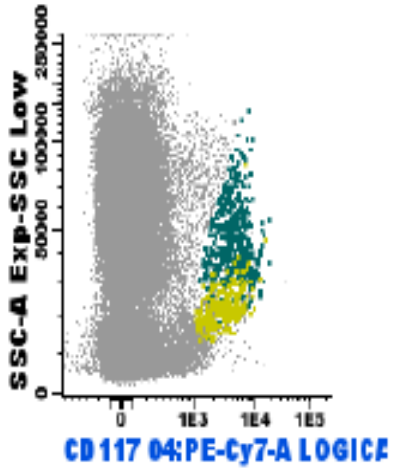


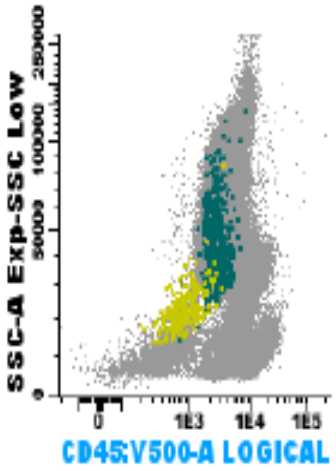




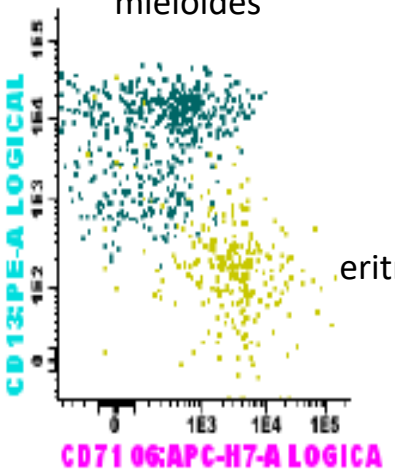
Evaluación de Células Precursoras

Precursores CD34(-) CD117(+)





mieloides



eritroides

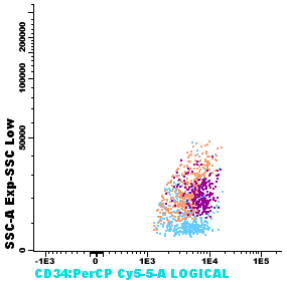
Alteraciones Fenotípicas en Progenitores

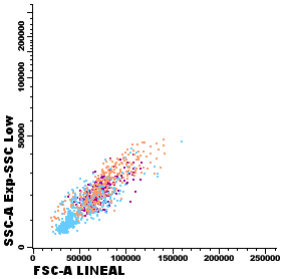
Progenitores mieloides:

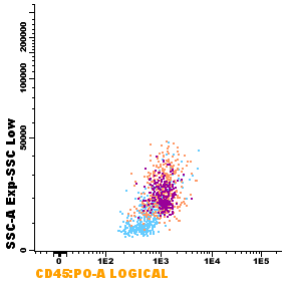
- Aumento porcentual de células CD34(+).
- Aumento porcentual de células CD34(-) CD117(+).
- Expresión de CD11b y/o CD15.
- Aumento de la subpoblación CD34(+) CD38(+) débil.
- Expresión alterada (ausencia/disminución/sobreexpresión) de CD13, CD33, CD45, CD117 o HLA-DR.
- Expresión de antígenos linfoides (TdT, CD5, CD7 y/o CD56).
- SSC anormal (granularidad).

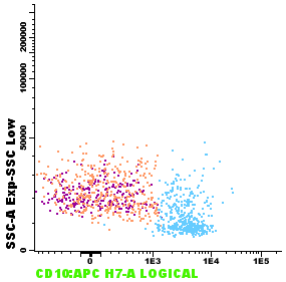
Progenitores CD34(+) linfoides:

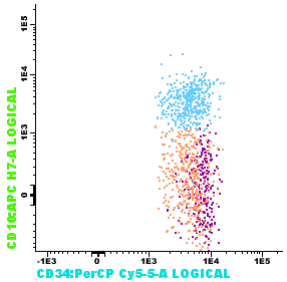
- Disminución relativa (% de CD19(+) CD10(+) en el gate CD34(+))
- Ausencia de expresión de CD79a citoplasmático.











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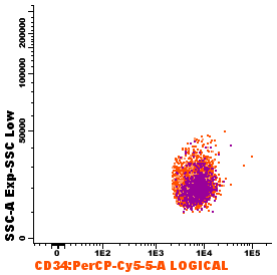
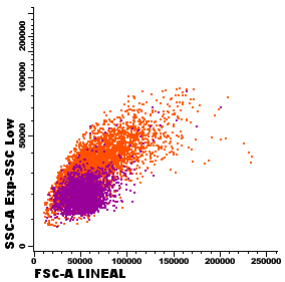
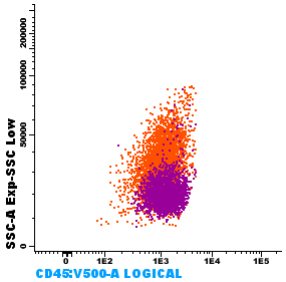
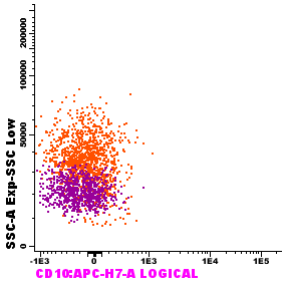
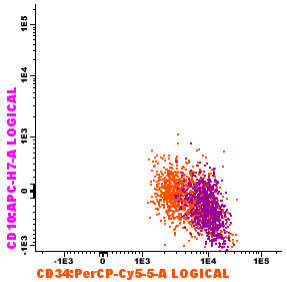










SMD

El porcentaje de precursores CD34+ linfoides se informa sobre el total de CD34+.

Se considera anormal <5% del total de las CD34+.

Cytometry Part B (Clinical Cytometry) 70B:63–70 (2006)

Increased Immature Hematopoietic Progenitor Cells $CD34^{+}/CD38^{dim}$ in Myelodysplasia

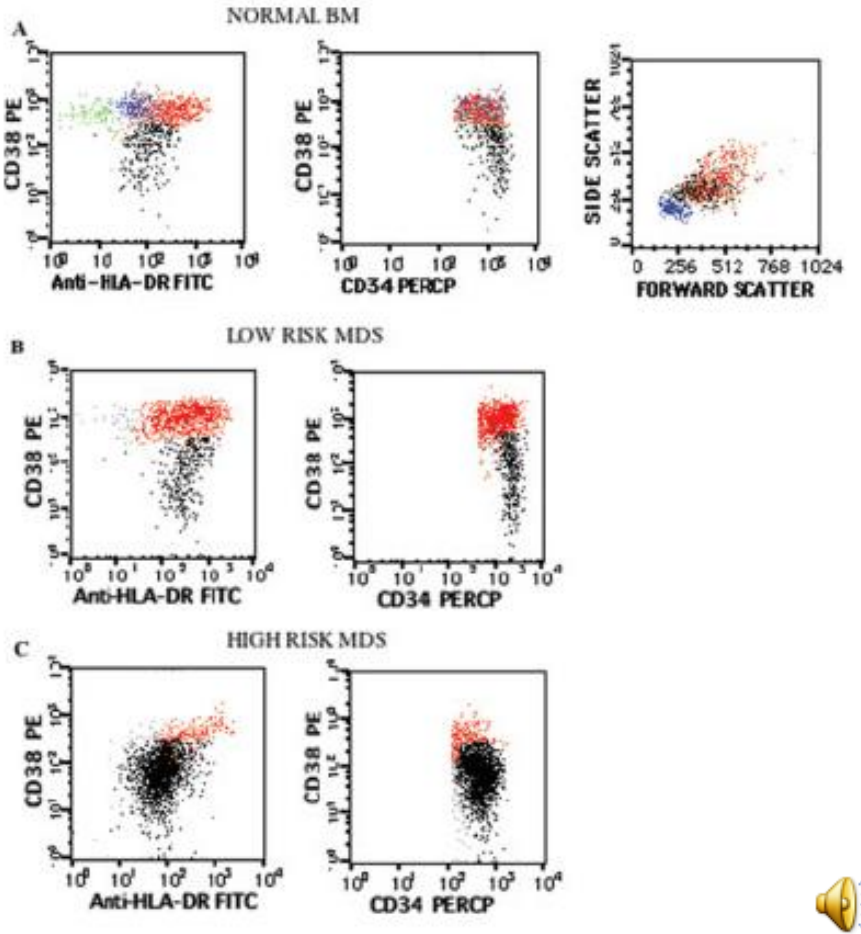
Mariela B. Monreal,* Maria L. Pardo, Miguel A. Pavlovsky, Isolda Fernandez, Claudia S. Corrado, Isabel Giere, Sandra Sapia, and Santiago Pavlovsky
FUNDALEU (Foundation to Fight Leukemia) and Centro de Investigacion Clinica A. Ocampo, Buenos Aires, Argentina

Background: Myelodysplastic syndromes (MDS) are clonal disorders affecting hematopoietic progenitor cells (HPC). Despite the relevance of clonal $CD34^{+}$ cells in developing MDS, only few studies analyze the phenotype of this cell population. The aim of this study was to evaluate phenotypic changes on HPC in MDS that could reflect abnormalities in the differentiation process of stem cells.

Methods: We analyzed the expression of CD38 and HLA-DR on $CD34^{+}$ cells by flow cytometry in 36 patients with MDS, as well as in healthy donors (n = 12) and patients with other hematological disorders: non-Hodgkin lymphomas and multiple myeloma, both in complete remission (CR) (n = 32); acute lymphoblastic leukemia in CR (n = 17); de novo acute myeloblastic leukemia (AML) at diagnosis (n = 22) and in CR (n = 37); and AML secondary to MDS at diagnosis (n = 19). Cases with available karyotype were grouped according to the International Prognostic Scoring System (IPSS).

Results: Compared to normal BM, the fraction of immature HPC, characterized as $CD34^{+bright}$, intermediate FSC/SSC, and $CD38^{dim}$, was significantly increased in high risk MDS and secondary AML, but not in low risk MDS, ($P < 0.001$, $P = 0.03$, and $P = 0.7$). De novo AML showed decreased immature HPC. High numbers of immature HPC correlated with higher IPSS risk groups ($P = 0.05$) and showed significant impact on disease progression ($P = 0.03$).

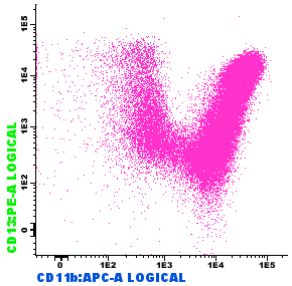
Conclusion: Our study confirms that evaluation of CD38 expression pattern on HPC is an easy and reproducible test that allows evaluating the immature subset of progenitor cells. Increased immature HPC in high risk MDS and secondary AML may reflect blocked differentiation of $CD34^{+}$ cells in these diseases. © 2006 International Society for Analytical Cytology

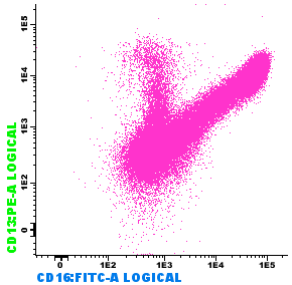


Alteraciones Fenotípicas en Serie Granulocito Neutrófilo

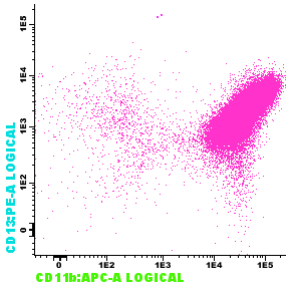
- Hipogranularidad (disminución de SSC, evaluado en relación al SSC de los linfocitos).
- Anormalidad en los patrones de maduración (CD16/CD13, CD13/CD11b).
- Expresión de CD117, HLA-DR.
- Expresión de antígenos linfoides (CD56).
- Expresión anormal de CD15, CD36, CD64, CD33 o CD45.
- Asincronismo en la expresión de CD10 y CD16.
- Disminución del porcentaje de granulocitos neutrófilos (en relación al de linfocitos).

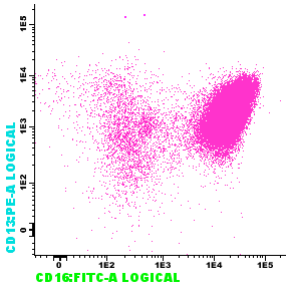
Normal

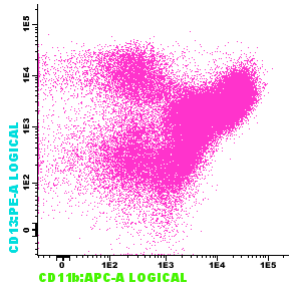


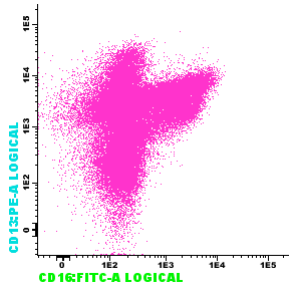


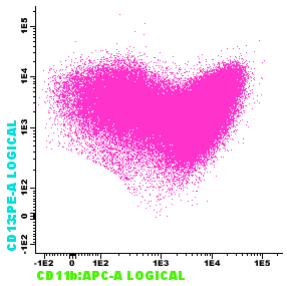
Alteraciones

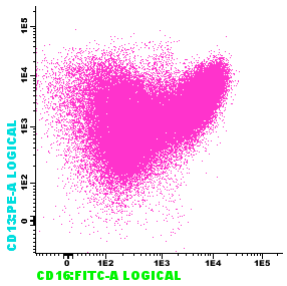


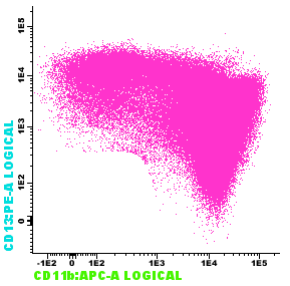


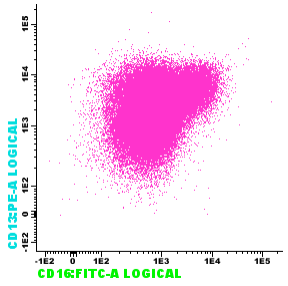








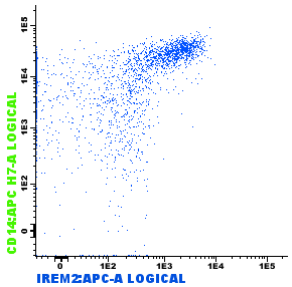


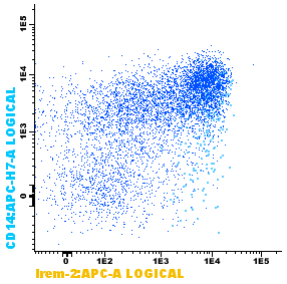


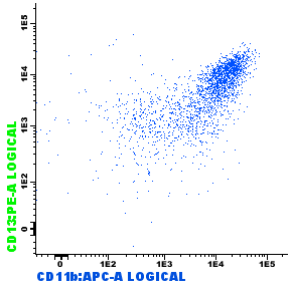
Alteraciones Fenotípicas en Serie Monocítica

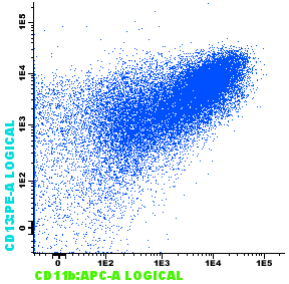
- Alteración en la granularidad (SSC).
- Expresión disminuída de CD45.
- Anormalidad en los patrones de maduración con CD35, HLA-DR, CD11b CD13, CD64, CD36, CD14, CD300e (IREM-2).
- Expresión de antígenos linfoides (CD56), con excepción del CD4.
- Ausencia de expresión de CD13, CD33.

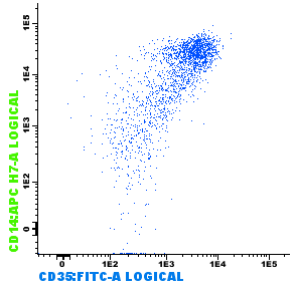
Normal

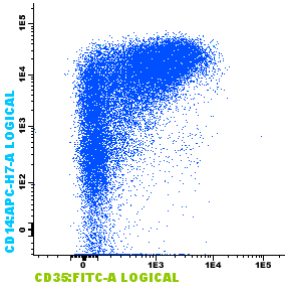




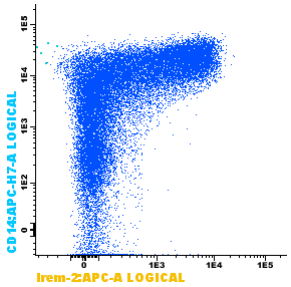


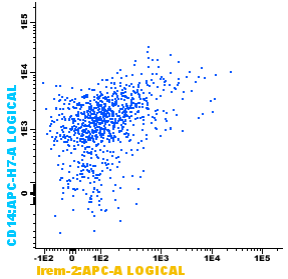


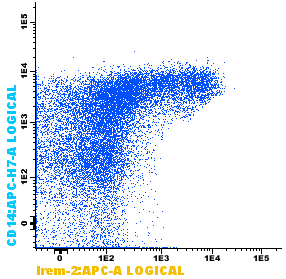


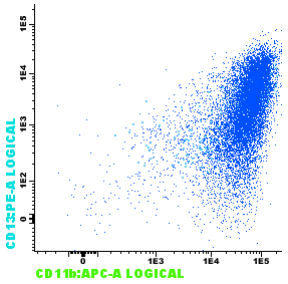


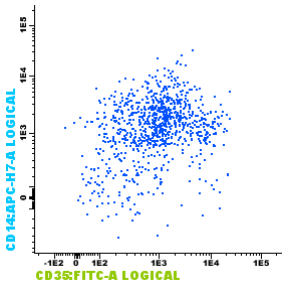
Alteraciones











- Bloqueo madurativo.
- Heterogeneidad de CD13 y CD11b.
- Asincronismo madurativo.

Alteraciones Fenotípicas en Serie Eritroide Nucleada

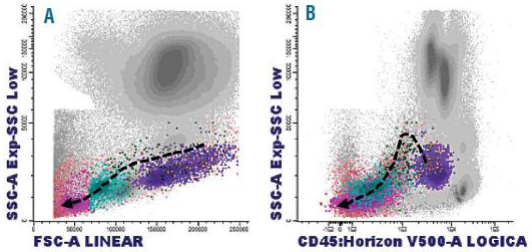
- Aumento porcentual de células eritroides nucleadas post lisis.
- Aumento de precursores CD34(-) CD117(+) CD105(+).
- Expresión anormal de CD71, CD36, CD235a (GlicA), CD105.

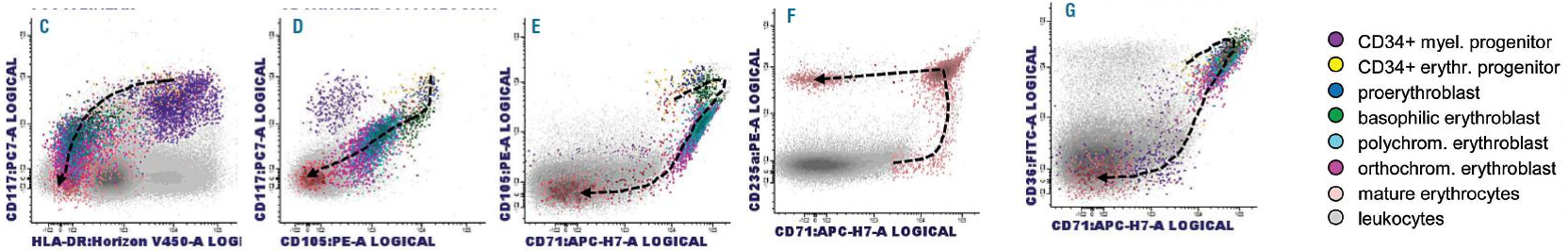
Linaje megacariocítico

- No se estudia por citometría de flujo.

Immunophenotypic analysis of erythroid dysplasia in myelodysplastic syndromes. A report from the IMDSFlow working group

Theresia M. Westers,[1] Eline M.P. Cremers,[1] Uta Oelschlaegel,[2] Ulrika Johansson,[3] Peter Bettelheim,[4] Sergio Matarraz,[5] Alberto Orfao,[5] Bijan Moshaver,[6] Lisa Eidenschink Brodersen,[7] Michael R. Loken,[7] Denise A. Wells,[7] Dolores Subirá,[8] Matthew Cullen,[9] Jeroen G. te Marvelde,[10] Vincent H.J. van der Velden,[10] Frank W.M.B. Preijers,[11] Sung-Chao Chu,[12] Jean Feuillard,[13] Estelle Guérin,[13] Katherina Psarra,[14] Anna Porwit,[15,16] Leonie Saft,[16] Robin Ireland,[17] Timothy Milne,[17] Marie C. Béné,[18] Birgit I. Witte,[19] Matteo G. Della Porta,[20] Wolfgang Kern[21] and Arjan A. van de Loosdrecht,[1] on behalf of the IMDSFlow Working Group





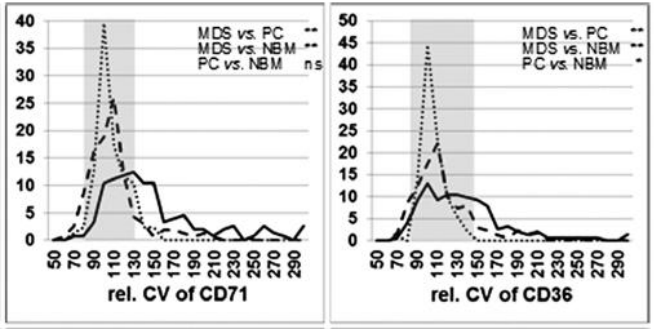
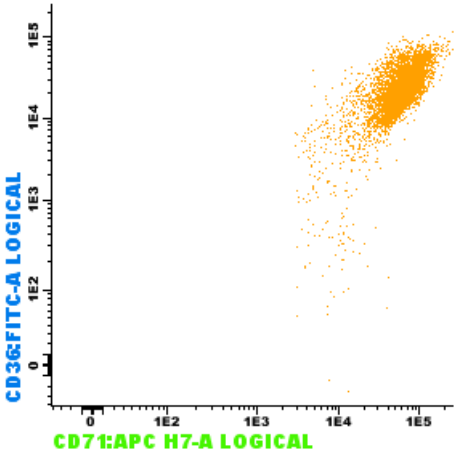
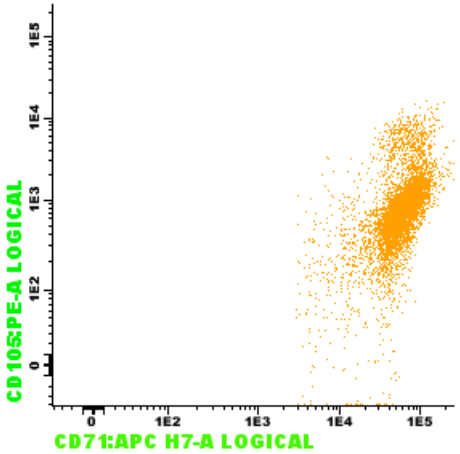


Table 4. Results of multivariate logistic regression analysis in learning cohort.

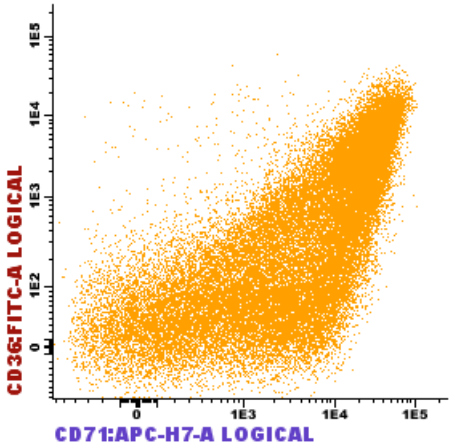
Parameter	odds ratio	95% CI	P
rel. CD36 CV	3.7	1.6 – 8.5	0.003
rel. CD71 CV	3.2	1.6 – 6.4	0.001
rel. CD71 MFI	2.2	1.1 – 4.5	0.033
rel. %CD117	1.7	0.92 – 3.2	0.084

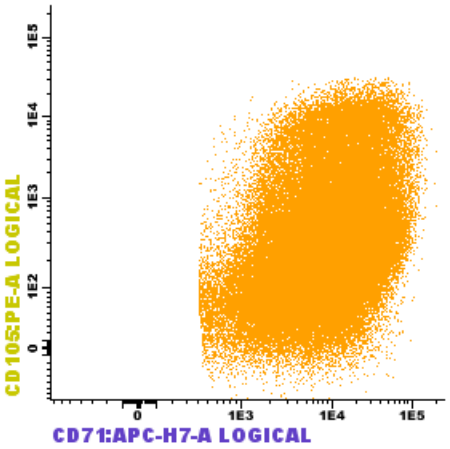
Markers entered in the analysis were relative CD36 MFI, CD36 CV, CD71 MFI and CD71 CV, and the relative percentage of CD117+ erythroid cells (%CD117). 272/535 cases were available for analysis of which 153 pathological controls and 119 MDS cases in the learning cohort; $P<0.001$). CI: confidence interval; CV: coefficient of variation; MFI: mean fluorescence intensity; P: P-value; rel.: relative.





Normal





Alteraciones

¿Qué informar?

Datos del paciente: Identificación del paciente, tipo de muestra, fecha de recepción.

Celularidad de la muestra:

- % de células CD34(+)
- % de células CD34(-)CD117(+)
- % de granulocitos.
- % de monocitos
- % serie eritroide
- % de otras células identificadas.

Descripción de las poblaciones analizadas:

- Fenotipo conservado o alterado de las células CD34(+). Presencia o ausencia de hematogonias.
- Patrón madurativo conservado o alterado en serie granulocítica, monocítica y/o eritroide.

Conclusiones:

- Si hay aumento de blastos.
- Si hay presencia de alteraciones que pueden ser vinculables con displasia uni o multilineal.

Cuidado con las muestras hemodiluídas

Otras causas de alteraciones fenotípicas

- Displasia multilinaje:
  - Malnutrición.
  - Falla multiorgánica.
  - Drogas inmunosupresoras o citotóxicas.
  - Abuso de alcohol.
  - Deficiencia de Vit B12 o Acido Fólico.
  - Pacientes HIV(+).
- Sobreexpresión de CD64 en sepsis.
- Ausencia de CD16 en granulocitos: descartar HPN.
- Ausencia de CD14 en monocitos: descartar HPN.

Importante conocer antecedentes del paciente para descartar otras causas.

Sistemas de Scores: FCSS (2003)

Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation

Denise A. Wells, Martin Benesch, Michael R. Loken, Carlos Vallejo, David Myerson, Wendy M. Leisenring, and H. Joachim Deeg

Table 3. Components of flow scoring system

Points	Myeloid abnormailities	Monocytic abnormalities
0	Appropriate CD45/SSC	Appropriate CD45/SSC
	Heterogeneous $CD11b^+$, $HLA\text{-}DR^-$	$CD11b^+$, heterogeneous HLA-DR
	Normal relationships of CD13 and CD16	CD13/CD16 coexpression
	$CD33^+$	CD33/CD14 coexpression
	$CD19/CD5/CD34/CD56/CD7^-$	$CD19/CD5/CD34/CD56/CD7^-$
	Synchronous shift to the left	
1	One of the following is present:	One of the following is present:
	Abnormal granularity	Abnormal granularity
	Abnormal decrease in CD45 expression	Abnormal CD11b or HLA-DR expression
	Presence of HLA-DR or lack of CD11b	Loss of CD13 or CD16
	Convex or abnormal relationship between CD13 and CD16	Presence of CD56
	Expression of CD56 on subpopulation of myeloid cells	Lack of CD33 or CD14
	Lack of CD33 expression (may be a normal variant)	
	Asynchronous shift to the left	
2	2-3 of the above abnormalities or presence of CD34 on myeloid cells or presence of lymphoid antigens on myeloid cells	2-3 of the above abnormalities or presence of CD34 on monocytes or presence of lymphoid antigens on monocytes (exception is CD4)
3	4 or more of the above abnormalities or 1 or more of the abnormalities plus presence of CD34 or lymphoid antigen expression on myeloid cells	4 or more of the above abnormalities or 1 or more of the abnormalities plus presence of CD34 or lymphoid antigen expression on monocytes

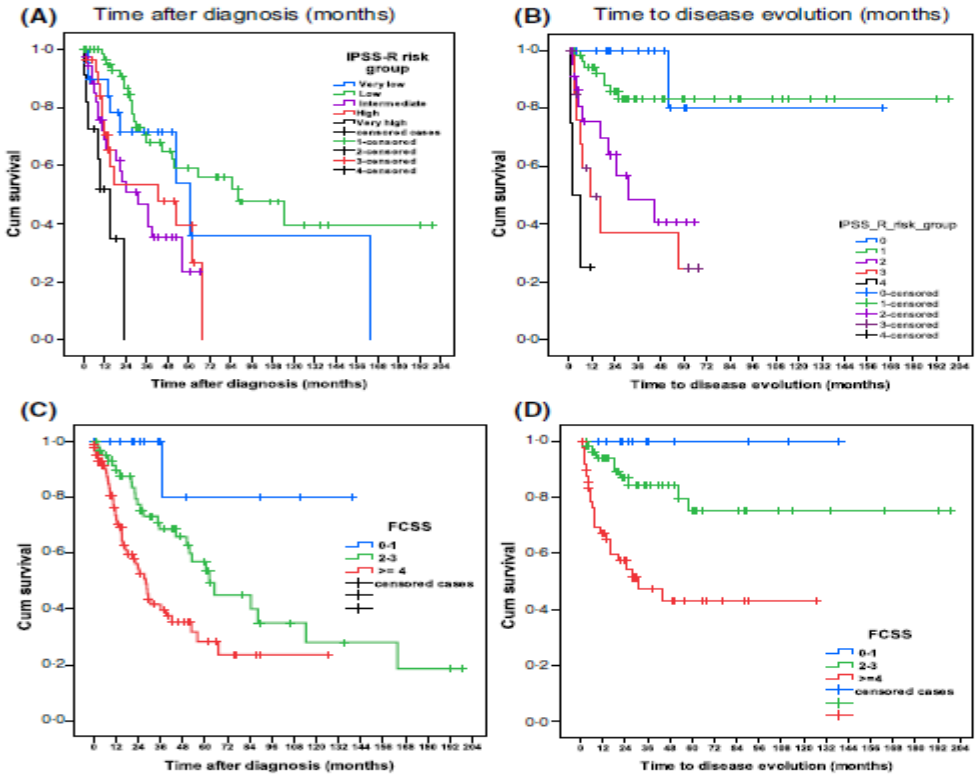
0-1 : normal ó con signos displásicos leves.
2-3 : evidencia displasia moderada.
≥ 4 : refleja displasia severa.

BLOOD, 1 JULY 2003 • VOLUME 102, NUMBER 1

bjh research paper

High flow cytometric scores identify adverse prognostic subgroups within the revised international prognostic scoring system for myelodysplastic syndromes

Correlación de FCSS con IPSS score e IPSS-R score



Blue line: IPSS-R very low risk
Green line: IPSS-R low risk
Purple line: IPSS-R intermediate risk
Red line: IPSS-R high risk
Black line: IPSS-R very high risk

Blue line: FCSS 0-1 point (s)
Green line: FCSS 2-3 points
Red line: FCSS ≥4 points

2014. British Journal of Haematology

Sistemas de Scores: Score de Ogata

2009

Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study

Kiyoyuki Ogata,[1] Matteo G. Della Porta,[2] Luca Malcovati,[2] Cristina Picone,[2] Norio Yokose,[3] Akira Matsuda,[4] Taishi Yamashita,[1,5] Hideto Tamura,[1] Junichi Tsukada,[6] and Kazuo Dan[1]

2012

Multicenter validation of a reproducible flow cytometric score for the diagnosis of low-grade myelodysplastic syndromes: results of a European LeukemiaNET study

Matteo G. Della Porta,[1] Cristina Picone,[1] Cristiana Pascutto,[1] Luca Malcovati,[1] Hideto Tamura,[2] Hiroshi Handa,[3] Magdalena Czader,[4] Sylvie Freeman,[5] Paresh Vyas,[6] Anna Porwit,[7] Leonie Saft,[7] Theresia M. Westers,[8] Canan Alhan,[8] Claudia Cali,[8] Arjan A. van de Loosdrecht,[8] and Kiyoyuki Ogata[2]

Hematológica, 2009 y 201

Table 3. Calculation of the flow cytometric score (FCM-score) for the diagnosis of low-risk MDS.

Cytometric Parameter	Cut-off values	Regression coefficient	Variable weighted score#
Myeloblast-related cluster size (%)*	≥2	2.59	1
B-progenitor-related cluster size (%)**	≤5	1.87	1
Lymphocyte to myeloblast CD45 ratio	≤4 or ≥7.5	1.76	1
Granulocyte to lymphocyte SSC ratio	≤6	2.31	1

**In all nucleated cells; **in all CD34+ cells; #a diagnosis of MDS is formulated in the presence of a FCM-score value ≥2.*

Valor ≥ 2: se asocia con alta probabilidad de SMD

Sistemas de Scores: Red Score (Mathis)

Leukemia (2013), 1–7
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www.nature.com/leu

ORIGINAL ARTICLE

Flow cytometric detection of dyserythropoiesis: a sensitive and powerful diagnostic tool for myelodysplastic syndromes

S Mathis[1,2,7], N Chapuis[1,2,7], C Debord[1], A Rouquette[3], I Radford-Weiss[4], S Park[2,5], F Dreyfus[2,5], C Lacombe[1,2], MC Béné[6], O Kosmider[1,2], M Fontenay[1,2] and V Bardet[1,2]

Parámetro	threshold	Puntos
CV CD71 (%)	< 80	0
	≥ 80	3
CV CD36 (%)	< 65	0
	≥ 65	2
Hb (g/dl)	>10.5 (F) o >11.5(M)	0
	≤10.5 (F) o ≤11.5 (M)	2

Valor ≥ 3: se asocia con SMD

Sistemas de Scores: Erytroid Score.

Implementation of erythroid lineage analysis by flow cytometry in diagnostic models for myelodysplastic syndromes

by Eline M.P. Cremers, Theresia M. Westers, Canan Alhan, Claudia Cali, Heleen A. Visser-Wisselaar, Dana A. Chitu, Vincent H.J. van der Velden,

Parámetro citométrico	Aberrancia	Valor
CV CD71	Aumentado	1
MFI CD71	Disminuído	1
CV CD36	Aumentado	1
% CD117 en eritroides	Aumentado o Disminuído	1

Theresia M. Westers,[1] Eline M.P. Cremers,[1] Uta Oelschlaegel,[2] Ulrika Johansson,[3] Peter Bettelheim,[4] Sergio Matarraz,[5] Alberto Orfao,[5] Bijan Moshaver,[6] Lisa Eidenschink Brodersen,[7] Michael R. Loken,[7] Denise A. Wells,[7] Dolores Subirá,[8] Matthew Cullen,[9] Jeroen G. te Marvelde,[10] Vincent H.J. van der Velden,[10] Frank W.M.B. Preijers,[11] Sung-Chao Chu,[12] Jean Feuillard,[13] Estelle Guérin,[13] Katherina Psarra,[14] Anna Porwit,[15,16] Leonie Saft,[16] Robin Ireland,[17] Timothy Milne,[17] Marie C. Béné,[18] Birgit I. Witte,[19] Matteo G. Della Porta,[20] Wolfgang Kern[21] and Arjan A. van de Loosdrecht,[1] on behalf of the IMDSFlow Working Group

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torti Foundation

Valor ≥ 2: se asocia a displasia eritroide asociada a SMD

Conclusiones I

- La muestra para el estudio de SMD por CF debe ser el aspirado de médula ósea.
- Evaluar todas las poblaciones presentes en MO, por lo menos las mayoritarias, con amplios paneles de Ac. Monoclonales.
- Los datos obtenidos por CF deben ser capaces de categorizar MO como normal o posiblementemente vinculable con displasia.
- La CF en SMD puede ser usada sólo como co-criterio diagnóstico.
- No existe un marcador diagnóstico específico para SMD.
- La presencia de las diferentes alteraciones fenotípicas en SMD es muy variable.

Conclusiones II

- Una única alteración no es significativa.
- Un mayor número de alteraciones muestran un mayor grado de especificidad diagnóstica y también se asocian con peor pronóstico.
- Pueden ser necesarios repetidos estudios en casos no concluyentes.
- Puede ser útil para monitorear el curso de la enfermedad tanto en pacientes de bajo riesgo no tratados como en los pacientes con tratamiento.
- Los SMD pueden coexistir con otras patologías como SLP, mastocitosis y HPN .
- Tener en cuenta que las citopenias pueden ser causa de otras patologías como leucemias agudas, anemias aplásicas y algunos SLPC como la Tricoleucemia.

MUCHAS GRACIAS

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